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Dizygotic Twin Sisters With Myelokathexis: Mechanism of its Neutropenia

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Dizygotic twin sisters were first found to have neutropenia at 1 year of age when evaluated for recurrent pulmonary infections. Since then they have remained neutropenic (0.05~0.5 × 10^9/l). Despite of their neutropenia, myeloid hyperplasia was evident on a marrow smear examination, and a number of cells were hypersegmented with fine interlobular bridging with chromatin strands and cytoplasmic vacuolation. Electron microscopy showed apoptotic cells with condensed nuclei and apoptotic bodies in the cytoplasm. Although life span, hydrogen peroxide production, phagocytosis, spreading, and chemotaxis of peripheral neutrophils were normal, the survival of bone marrow neutrophils in both infants was markedly decreased when compared with that of normal bone marrow neutrophils. During the bone marrow culture apoptotic neutrophils were observed at an earlier stage in both patients than in normal controls, biochemically and morphologically. Morphology of bone marrow neutrophils in both patients resembled that of cultured control bone marrow neutrophils. Peripheral neutropenia and appearance of characteristic neutrophils in the bone marrow in myelokathexis are considered to be an expression of apoptosis of bone marrow neutrophils. Am. J. Hematol. 62:106–111, 1999. © 1999 Wiley-Liss, Inc.

Key words: myelokathexis; neutropenia; apoptosis; hypogammaglobulinemia; neutrophil function

INTRODUCTION

Myelokathexis is a rare form of chronic neutropenia first reported by Zuelzer [1] in 1964. A cytological feature of the bone marrow neutrophils is the abundance of mature cells with dense and hypersegmented nuclei, containing cytoplasmic vacuoles suggestive of degeneration (apoptosis). The pathogenesis of the peripheral neutropenia and abnormal bone marrow neutrophils, however, still remains to be elucidated. Only 18 patients [1–12] have been reported so far in the world literature including the present two subjects. We report twin sisters with myelokathexis, whose chronic neutropenia is considered to be an expression of apoptosis of bone marrow neutrophils.

CASE REPORTS

Dizygotic twin sisters are 5-year-old with a history of recurrent respiratory infections beginning at 6 months of age. They are the second and the third of three children. The parents and brother are healthy and non-neutropenic, and none of the family members or relatives are similarly affected.

The first-born girl was diagnosed as having ventricular septal defect and pulmonary atresia with major aorticopulmonary collateral arteries. Both infants received two doses of oral polio vaccine, DPT, measles vaccine and BCG, each without incident.

At 1 year of age they were equally found to have neutropenia, and since then, absolute neutrophil counts of 0.3 ~ 0.5 × 10^9/l have persisted, with expected neutrophilia on the occasions of bacterial infections. The neutropenia was not cyclic in nature, and serial counts. Respiratory infections have been associated with sputum

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TABLE I. Peripheral Blood Counts and Bone Marrow Parameters

Parameters	Twin 1	Twin 2
Peripheral blood		
White Blood Cells ($\times 10^9$/l)	2.00	1.40
Polymorphonuclear	0.48	0.06
Lymphocytes	1.26	1.22
Monocytes	0.26	0.04
Basophils	0.00	0.04
Eosinophils	0.00	0.04
Hemoglobin	17.0	10.9
Platelets ($\times 10^9$/l)	186	642
Bone marrow		
M:E ratio	5.3	2.4
NCC* ($\times 10^9$/l)	186	168.5
Myeloblasts	1.1%	1.1%
Promyelocytes	3.3%	4.7%
Myelocytes	8.6%	7.0%
Metamyelocytes	8.7%	10.8%
Bands	5.6%	2.8%
Polymorphonuclears	29.9%	22.8%
Eosinophils	1.9%	1.8%
Basophils	0.3%	0.0%
Monocytes	3.2%	1.8%
Lymphocytes	26.0%	30.0%

*NCC: nucleated cell count.

TABLE II. Immunological Studies

Parameter	Twin 1	Twin 2	Normal range (means ± 2SD)
Immunoglobulin (mg/dl)			
IgG	405	458	940 ± 184.1
IgA	21	29	185.1 ± 61.4
IgM	191	190	83.3 ± 21.3
Peripheral blood Immmunophenotype (%)			
CD3	58.1	68.3	59.8 ± 13.1
CD4	45.1	52.3	40.5 ± 16.8
CD8	14.8	16.8	22.6 ± 10.3
CD4/8	3.06	3.11	1.9 ± 1.2
CD20	3.8	6.6	8.2 ± 8.6
Sm-IgG	1	1	2.6 ± 2.6
Sm-IgM	3	3	8.8 ± 8.6
Sm-IgA	0	0	2.6 ± 2.6
Sm-IgD	2	3	4.2 ± 2.5
Lymphocyte mitogenic response (Stimulation Index)			
Phytohemagglutinin	254	276	20~
Concanavalin A	274	155	20~

cultures positive for Haemophilus influenzae and Streptococcus pneumonia. Developmental milestones have been normal in both infants.

At 3 years of age, they were admitted for evaluation of neutropenia. Physical findings of both sisters were essentially negative except for a minimal cyanosis of lips and grade 2 systolic murmur along the left sternal border of the first-borne infant. They had no warts on the skin. Laboratory studies to delineate peripheral blood and bone marrow characteristics were summarized in Tables I and II. Bone marrow cytology was normocellular in both patients. Peculiar findings were hypersegmentation of nuclei connected with long fine strands and cytosolic vacuolization of neutrophils (Fig. 1). Electron microscopy confirmed the presence of abnormal mature neutrophils in the bone marrow in both patients. A few abnormal mature neutrophils appeared to be apoptotic with condensed nuclei and apoptotic bodies in the cytoplasm. Many apoptotic neutrophils were observed in bone marrow biopsy specimens; these were often phagocytized by macrophages (Fig. 2). There was no maturation arrest of the granulocyte series, but rather an increase of mature neutrophils. Colony forming units of the bone marrow cells were within normal limits in both subjects. Serum antineutrophil antibodies were negative when examined by using granulocyte immunofluorescence test (GIFT) [13].

Both infants were temporarily treated with granulocyte colony-stimulating factor (G-CSF), which increased neutrophil counts 24 hr later (0.3×10^9/l to 2.478×10^9/l in patient 1 and 0.05×10^9/l to 1.566×10^9/l in patient 2. Morphology of neutrophils of the patients was normal 24 hr after the treatment. Treatment with sulfamethoxazole-trimethoprim was started for recurrent infections at 3 years of age. A remarkable reduction of the rate of respiratory infections was noted.

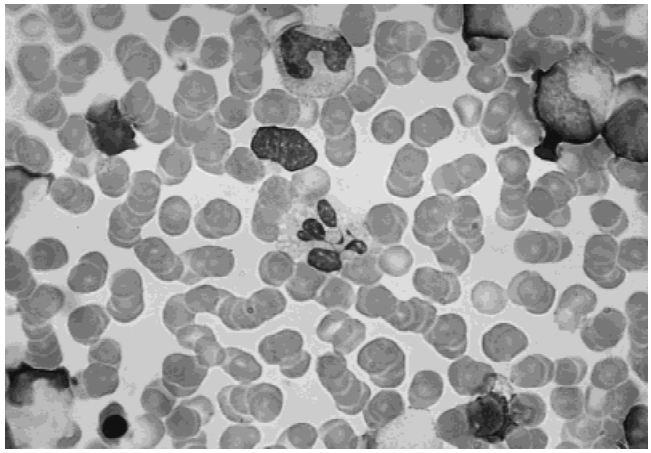

Fig. 1. A bone marrow aspirate from twin 1 showed an accumulation of mature neutrophils with cytoplasmic vacuoles and dense, pyknotic nuclear lobes with interconnecting filaments (Wright Giemsa stain; original magnification ×1,000).

METHODS

Isolation of Neutrophils

Informed consent was obtained from the parents of patients or age-matched normal controls. Normal bone

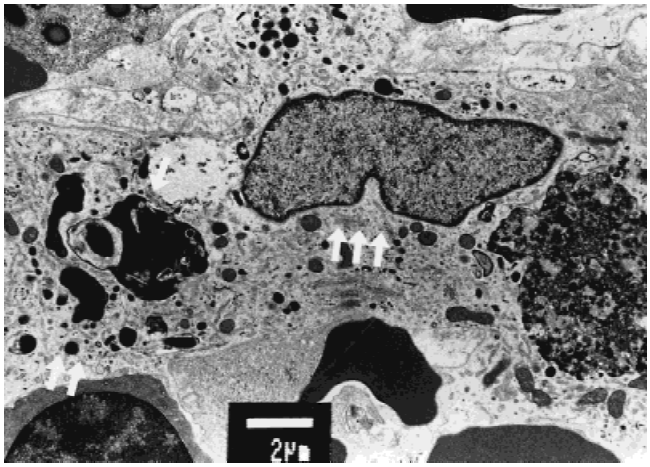

Fig. 2. An electron micrograph of a formalin-fixed bone marrow biopsy specimen from twin 1 showed an apoptotic neutrophil with condensed nuclei (↓) and apoptotic bodies (↓↓) in the cytoplasm by phagocytosis of macrophage. The same finding was observed in twin 2. ↓↓↓, nuclei of macrophage.

marrow cells for this study were obtained from hematologically normal pediatric patients ranging in age from 3 to 5 years who underwent the procedure for diagnostic purpose. Neutrophils were isolated by dextran sedimentation and Ficoll–Hypaque gradient centrifugation from heparinized peripheral blood and bone marrow aspiration of patients or controls. Residual erythrocytes were hypotonically disrupted with distilled water [14]. Viability was more than 99% as judged by trypan blue exclusion, and purity was more than 95% and 85% for peripheral and bone marrow neutrophils, respectively.

Neutrophil Function Tests

According to our method [15], we measured simultaneously both phagocytosis and production of hydrogen peroxide of neutrophils in whole blood by using flow cytometry. Chemotactic assay was also determined by the modified Boyden method [16]. Neutrophil spreading assay was modified as described by Crowley et al. [17] and observed by using a phase-contrast inverted microscope. Neutrophil life span assay was carried out by using a culture of neutrophils incubated in RPMI 1640 medium with 10% fetal bovine serum [18]. Viable cells were counted with a trypan blue solution. Colony forming assay was performed by the Terry Fox Laboratory kits.

Detection of DNA Fragmentation

Isolated bone marrow neutrophils (2×10^6 of cells) from the patients and a control were incubated in RPMI 1640 medium with 10% fetal bovine serum for DNA extraction. The cells were washed in phosphate-buffered saline, pH 7.4, to remove traces of medium at the time of 0 hr and 24 hr of culture. DNA extraction was performed by using a Sepa Gene kit (Sanko Junyaku Chemical Co., Tokyo, Japan). After remaining RNA was digested with 1 μg/ml RNaseA, a total of 2×10^6 neutrophils was loaded per a lane in 1.6% agarose gel containing ethidium bromide. After electrophoresis, the gel was photographed under ultraviolet light.

Quantitiation of Apoptotic Cells

Apoptotic cultured neutrophils were measured by staining with propidium iodide (100 μg/ml) by flow cytometer [19]. Briefly cell pellets by centrifugaiton were treated with 2 ml of cold 70% ethanol and fixed at −20°C for more than 2 hr. After washing, the cell pellets were suspended in 0.5 ml phosphate-buffered saline. Then, 500 ml RNase (1 mg/ml) and 1 ml propidium iodide (100 μg/ml) were added. The mixtures were kept at 4°C in the dark until examined. Fluorescence of individual cells was measured with a flow cytometer (EPICS-C; Coulter Corp, Hialeah, FL).

RESULTS

Hydrogen peroxide production, phagocytosis, chemotaxis, and spreading of peripheral neutrophils were normal (data not shown). Survival of peripheral neutrophils in both patients were slightly shortened but within normal ranges of control neutrophils (data not shown). However the survival of bone marrow neutrophils in both infants was remarkably decreased, compared with that of normal bone marrow neutrophils (Fig. 3). During culture, apoptotic neutrophils were observed earlier in both patients than in normal controls (Fig. 4, left). This finding was confirmed quantitatively by increased apoptotic neutrophils of patients' neutrophils compared with controls at 12 hr, 24 hr, and 48 hr of culture by flow cytometric analysis (Fig. 5). We also confirmed this finding by DNA fragmentation. Cultured neutrophils for 24 hr from both patients showed DNA fragmentation, while controls did not (Fig. 6). Morphology of bone marrow neutrophils in both patients (Fig. 4, right) resembled that of cultured bone marrow neutrophils of controls.

DISCUSSION

The twin sisters reported here display myelokathexis characterized by hypogammaglobulinemia and a bone marrow with increased granulocyte reserves and unique morphology. Eighteen patients have been described in the literature [1–12]. Although some patients may have this syndrome as the result of a spontaneous mutation, 12 of 18 including our cases appear to be inherited, of which inheritance pattern remains unclear. In six patients there is an association of warts, hypogammagloblinemia,

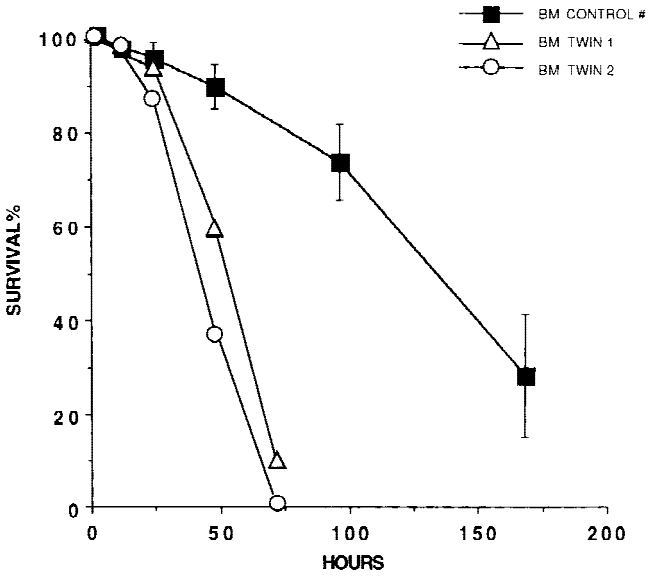

Fig. 3. Survival of bone marrow neutrophils. Neutrophils were incubated at 1 × 10^6 cells/ml in RPMI containing 10% fetal bovine serum and then evaluated for viable cells at various time points. Three non-neutropenic children were used as controls. The marrow tests of each patient were performed twice. Data were averages of duplicate experiments. A bar indicates a standard error of the mean.

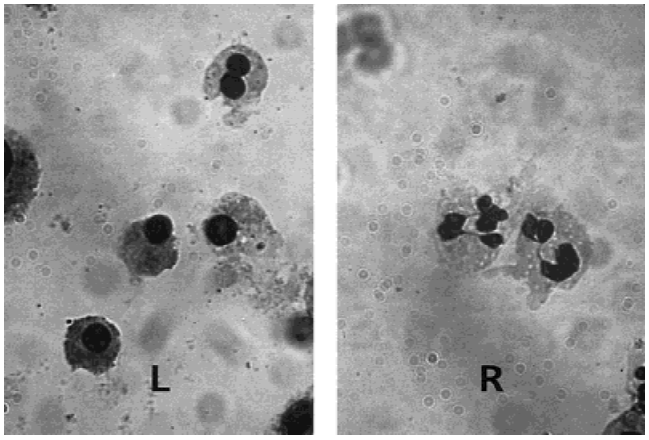

Fig. 4. Bone marrow neutrophils from twin 1 (left) and a control (right) were cultured for 24 hr and 48 hr, respectively, and then stained with Wright Giemsa. Left: Cells with high condensed and fragmented nuclei were considered apoptotic. Right: Morphology of cells resembled those of patient bone marrow PMNs.

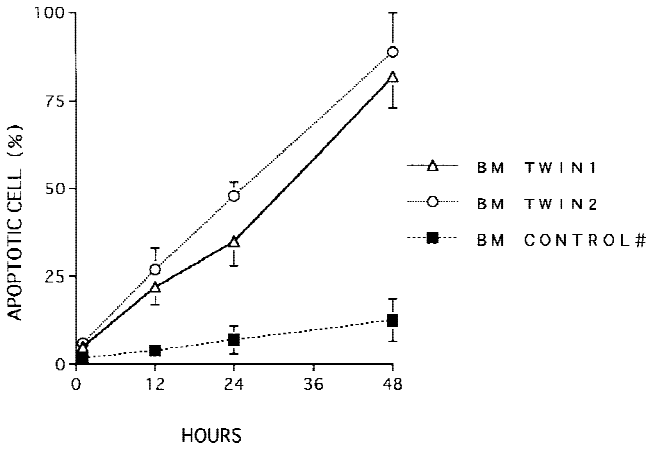

Fig. 5. The percentage of apoptotic cells was estimated by flow cytometry as described in Methods at the indicated times. Data were averages of triplicate experiments. A bar indicates a standard error of the mean. Three non-neutropenic children were used as controls.

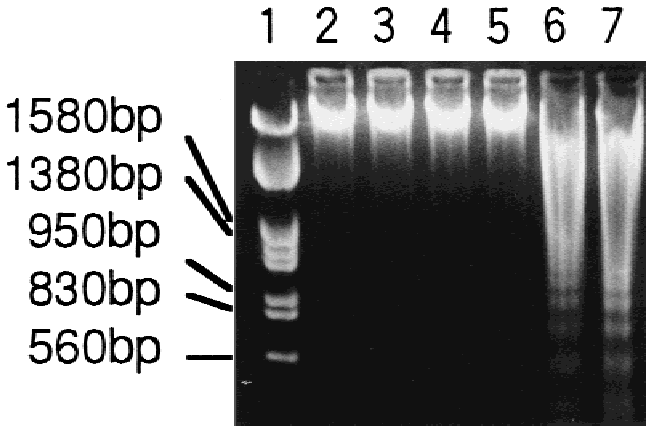

Fig. 6. Agarose gel electrophoresis of DNA extracted from Ficoll-separated bone marrow PMNs before (t0) and after (t24) incubation in RPMI 1640 medium containing 10% fetal bovine serum, for 24 hr at 37°C. Ethidium bromide staining was photographed in ultraviolet light. Lane 1: DNA molecular-weight markers. Lanes 2, 3, 4: Neither the control nor both patients show any evidence of low-molecular-weight DNA at 0 hr. Lane 5: The control did not show any evidence of low-molecular-weight DNA at 24 hr. Lanes 6, 7: Both patients clearly demonstrated a characteristic fragmentation at 24 hr.

and recurrent bronchopulmonary infections with myelokathexis: WHIM syndrome [12]. Because the twin sisters were dizygotic and no family members were similarly affected and have no warts, our cases appear to be of recessive trait which is different from the WHIM syndrome.

The term myelokathexis (neutropenia with kathexis, i.e., retention of bone marrow neutrophils) was applied to this syndrome [1], but the mechanism of this phenomenon is still unclear. The survival of patients' bone marrow neutrophils was extremely shortened when compared to controls and grossly comparable to that of normal peripheral neutrophils. During culture, apoptotic bone marrow neutrophils in both patients were observed earlier than in controls. The DNA fragmentation in electrophoresis and increased apoptotic cells with DNA staining confirmed that this observation can be explained

by an expression of apoptosis of bone marrow neutrophils. Morphology of bone marrow neutrophils in patients resembled that of cultured control bone marrow neutrophils. Appearance of characteristic bone marrow neutrophils of the patients may be related to a primary morphologic change of apoptosis of bone marrow neutrophils. However, a possibility could not be excluded that if the defect in myelokathexis resides in the inability of bone marrow neutrophils to be mobilized from the bone marrow into the peripheral blood, these neutrophils would be retained in the bone marrow and destined to be apoptotic cells, which could not be seen in a normal marrow.

Raza et al. [20] showed that the paradox of pancytopenia despite hypercellular marrow in myelodysplastic syndrome (MDS) may be explained by extensive intramedullary death. Myelokathexis, in contrast, is characterized by isolated neutropenia associated with myeloid hyperplasia. The difference in syndromes may be due to that of stages of cell death. In myelokathexis it occurs in the mature stage of myeloid development, whereas in MDS it takes place in the early stages of trilineage development. Interestingly, two reports [21–22] described cases of myelokathexis-like variant of MDS. It is therefore proposed that a maturation of myeloid cell lineage may be genetically accelerated compared to that in a normal bone marrow, resulting in increased apoptotic neutrophils in the bone marrow and consequent peripheral neutropenia.

There have been several reports [17,23] that either G-CSF or granulocyte-mucrophage colony-stimulating factor (GM-CSF) prolongs neutrophil survival in normal subjects and inhibits neutrophil apoptosis. A recent study [11] described that once a patient with myelokathexis was treated with GM-CSF, normal-appearing neutrophils increased with disappearance of characteristic neutrophils. It is therefore likely that G-CSF or GM-CSF inhibits apoptosis of characteristic bone marrow neutrophils of this syndrome, allowing normal neutrophils mobilized from the bone marrow to circulate in peripheral blood. Another possibility is that these cytokines induce changes in the adhesive properties of patients' neutrophils, which could contribute to the mobilization of progenitor cells from the bone marrow to the peripheral blood. Interestingly, the prolonged administration of cytokines such as GM-CSF [24] and G-CSF [7] resulted in a reduction of the incidence of infection associated with the increased immunoglobulin levels. This increase could be an indirect effect of GM-CSF and G-CSF, i.e., via stimulation of cytokine production by mononuclear cells, and it is possible that the rise in peripheral blood lymphocyte count is related to this phenomenon.

Although the pathogenesis of this disorder is unclear, it seems likely that the abnormality of these cytokine networks cause increased apoptosis of neutrophils and decreased production of immunoglobulin with lymphopenia.

In conclusion, 5-years-old twin sisters with myelokathexis represented neutropenia, characteristic morphology of bone marrow neutrophils and hypogammaglobulinaemia. Peripheral neutropenia and appearance of characteristic neutrophils in the bone marrow is considered to be an expression of apoptosis of neutrophils.

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